Development of Hybrid Honey Bees

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Abstract

The history and development of the artificial insemination technique into a usable and essential tool for breeding honey bees are discussed, followed by the hybrid honey bee program carried and developed by the U.S. Department of Agriculture and Canada over 20 years. The breeding methods used are included along with selected examples of observed stock variability, problems in holding or maintaining inbreds, and how industry has used and benefited from the work. A look is projected to the future, emphasizing the need for methods using less intensive inbreeding and the development of queen and sperm banks.

Key words:
Hybrid honey bee, Sperm banks, Inbreds, Artificial insemination

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Historical Review

Evolution of a concept

Considerable genetic variability exists between the races and strains of bees in the Old World. Most of these differences are the result of natural selection over a long period. Honey bees in the New World are the resulting complex of a large number of importations of stock from other parts of the world. Dr. W. C. Roberts,[1] who worked on the hybrid honey bee production program, suggests that because controlled natural matings of honey bees are not possible, the new stocks mated with the existing stocks and produced hybrids with the old and new imported stocks. Later these appeared to degenerate and new strains were imported and their superiority proclaimed. Thus, beekeepers were unwittingly hybridizing several races of bees without realizing that their superior colonies were hybrids rather than pure races (*17*).[2]

In the mid-1930's the U.S. Department of Agriculture (USDA) studied the causes of queen supersedure using 5,000 packages and queens in commercial yards. Differences in stocks and their capacity to produce were strongly evident in these studies. When the Madison, Wis., bee research laboratory was started in 1938, package bees and queens from several sources were used, and the differences in stocks further emphasized the variability present in commercial strains of honey bees. Interest in stock variability stimulated a stock testing program at Madison. Detailed records of performance of colonies were made with primary emphasis on honey production.

The technique of artificial insemination, developed through the work of L. R. Watson, W. J. Nolan, and H. H. Laidlaw, was perfected by O. Mackensen and W. C. Roberts. L. R. Watson in 1926 (*24*) first described a technique of artificial insemination of queen honey bees. W. J. Nolan in 1929 (*11, 12*) simplified the Watson technique. In 1932, Laidlaw (*6*) described a hand-mating method of artificial insemination and in 1949 (*7*) developed a new apparatus for breeding. He described how to insert the syringe beyond the valvefold so semen could be placed into the median oviduct.

Roberts and Mackensen (*15*) developed a method of queen insemination in the early 1940's that perfected the technique as a usable and essential tool for breeding honey bees. They also used a larger volume of semen (*10*). Without the technique of artificial insemination, controlled breeding of honey bees is virtually impossible because normal mating occurs with numerous unknown drones at some distance from the colony (*21, 25, 26*).

With the development of a usable technique of artificial insemination, hybrid bees became a reality. In 1943, the first artificially inseminated hybrid queens were produced at the Baton Rouge, La., laboratory and were tested at the Madison laboratory in bee colonies. Four of six queens produced worker offspring throughout a 4-month test. The Madison laboratory tested 30 queens in 1944 and 62 queens in 1945 artificially mated at Baton Rouge. In 1946, the Madison laboratory had 200 test colonies, and 160 of them were headed by artificially inseminated queens. These queens performed as well as naturally mated queens throughout 12 months in full-strength colonies.

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[1]Entomologist, formerly with Agricultural Research Service, U.S. Department of Agriculture.

[2]Italic numbers in parentheses refer to Literature Cited, p. 11.

In 1947, 490 queens were inseminated at Madison, and 395 laying queens resulted—over 80 percent, which is higher than the level of success normally expected for naturally mating virgins in this locality (*17*). The test colonies in 1947 produced an average of 96 pounds of honey per colony, and the highest producing line—a three-way hybrid stock headed by artificially mated queens—yielded 144 pounds of surplus honey.

To get the full advantage of hybrid vigor, both a hybrid queen and hybrid bees should be used in the same colony; this means at least a three-way hybrid. Soon, a four-way hybridization system was used (*15*). In 1963, E. R. Harp of the Madison laboratory began the use of a five-way hybrid to obtain a three-way hybrid queen from which to graft. The three-way hybrid queen produced more quality brood for production of the final hybrid. Harp recently produced a six-way hybrid using four-way breeding queens. What occurred in later years was a three-way hybrid that dropped in quality; additional lines were added to improve the hybrid until a six-way cross evolved. A two-way hybrid queen, if a true hybrid between unrelated stocks, should produce all the quality brood needed under normal conditions.

Improved honey production by genetically controlled stock became possible; inbred selections tested in hybrid combinations in full-strength colonies through 12 months provided limited selection. Inbred lines were lost from time to time as well as some of the most highly productive hybrids. One, the (ZX) (ME)[3] hybrid developed in 1955 at Madison, was exceptionally productive and possessed many other desirable characteristics. This stock outproduced all stocks tested against it. Although the hybrid has undergone considerable changes through the years because of loss and reconstitution of component inbreds, it has maintained a distinction to the present day as the (BZX) (DM) hybrid.

Production of hybrids and inbreds

The general system of breeding honey bees resembles that of hybrid corn production—a period of inbreeding and selection precedes intensive study of various inbred combinations used to make hybrid parental stocks from which hybrid seed is produced. With honey bees, inbreds are produced from several common stocks, crossed, and tested for their performance in hybrid combinations.

In 1944, findings showed that when a virgin queen is anesthetized with CO_2 for 10 minutes on 2 or 3 successive days and revived, she will start to lay eggs in a few days (*8*). The CO_2 treatment causes her to lose the instinct to mate and she becomes a drone layer because the unfertilized eggs hatch and develop by parthenogenesis into normal males or drones. The unfertilized drone-laying queen can be confined in a small screened mailing cage with five attendant bees after she has produced a quantity of drone brood. Thus confined, unable to perform normally, her ovaries again shrink so that for all practical purposes she is a virgin.

Egg laying should be completely stopped before insemination. Proper care must be given during caging, including the giving of a drop or two of water daily. When her drones reach maturity, she can be artificially inseminated with their sperm. Genetically, this is self-fertilization because the drones represent the queen's gametes. This technique has been found useful in the production of inbred lines. The progeny often exhibit a low brood viability caused by homozygous, lethal sex alleles (*9*). The queens producing the better brood are selected for further breeding.

A number of systems are used to produce inbred lines (*3*); the self-insemination method just described is not a standard bee breeding procedure. It gives a rapid rate of inbreeding, which may or may not be desirable. Other methods such as brother-sister, aunt-nephew, uncle-niece, and continuous cousin matings are more generally practiced in bee breeding. The slower means of inbreeding are generally more desirable when holding inbred stocks over many years.

Testing of inbreds was never a primary effort though some was done. There was selection among inbred lines for such qualitative traits as color, temper, or anything else that was measurable in the individual bee and thought important. The procedure adopted was to select three or four inbreds for study and test all the other lines for ability to produce good hybrids with these rather than to test all possible combinations of inbreds, which would be prohibitive.

Once two or more inbreds are crossed by artificial insemination, especially if they are unrelated,

[3]Inbred lines were arbitrarily identified by means of letters; combinations of such inbreds then denoted the hybrids. Lines Z and X in this hybrid were carried by the queen mother who was then mated to drones of M×E inbred lines in the drone mother.

vigor is expressed and the resulting hybrid can be tested and characterized. From 1946 to 1966 some 200 colonies of hybrid bees in four outyard locations were used to test, select, and breed.

Industry, USDA, and Canadian coordination

On November 24, 1947, the USDA's Bureau of Entomology and Plant Quarantine entered into a cooperative relationship with the Ohio Honey Bee Improvement Cooperative Association with headquarters at Columbus, Ohio, for the "purpose of fostering production and distribution of honey bee stock." A formal memorandum of understanding between the two organizations was signed.

The Ohio Honey Bee Improvement Cooperative Association (HBICA) was a group of Ohio honey producers who organized and incorporated on July 14, 1947, under the laws of Ohio as a nonprofit organization.

Dr. Otto Mackensen of the Southern States Bee Culture Laboratory, Baton Rouge, La., was in charge of the technical phases of the work. He was assisted by Dr. W. C. Roberts of the North Central States Bee Culture Laboratory at Madison.

By the time the cooperative agreement was initiated, the Bureau had already made considerable progress in selecting and breeding superior lines of bees. Cooperation included wide-scale testing of such lines of stock under commercial conditions and providing a channel for making those lines that were found most desirable available to the industry.

The queens produced for the tests were sold to honey producers who were equipped to make such tests and who agreed to submit reports for proper evaluation of the performance of the stock. No attempt was made to rear queens for general sale to honey producers.

The new Cooperative called for rearing 4,000 to 5,000 queens a year for sale to cooperators for testing under practical honey-producing conditions in different parts of the United States. These queens were from selected lines or hybrids between these lines. The program further provided for the release of breeder queens from any line or hybrid combination that definitely showed superiority under tests.

The queen-rearing phases of this project were centered on the islands at the western end of Lake Erie where isolated matings of queen bees were assured. Initially the work centered on Kelleys Island, Ohio; finances compelled the Cooperative to virtually close the work on the island by 1951.

In 1952, the project was better off financially but not enough to reopen Kelleys Island and conduct the queen rearing, selling, and testing program on the scale formerly anticipated. Thus, the Cooperative decided to award an exclusive contract to their queen breeder, J. G. Rossman of Moultrie, Ga., to rear, sell, and ship four-way hybrid queens to be known as Island Hybrid Queens. Orders went directly to Rossman through the Co-op. Rossman, in turn, paid a royalty per queen to the Co-op.

In September 1952, the Department of Agriculture of the Ontario Agricultural College, Guelph, Ontario, through Prof. G. F. Townsend, offered the use of its facilities on Pelee Island for the propagation of test queens. It received all inbreds raised at Madison for crossing and propagating. All queens raised on Pelee Island were available without cost to Guelph and Ottawa and to the USDA bee research laboratories for testing.

When the Kelleys Island and Pelee Island facilities were developed, test queens were naturally mated on the islands rather than artificially inseminated. Out of 573 queens artificially inseminated either once or twice in previous years, 66 percent became laying queens compared with 64 percent laying queens from 2,123 cells put out for naturally mated queens on Kelleys Island. The Kelleys Island facilities were disbanded in October 1954, and the equipment was sold to Rossman in Georgia. On December 26, 1963, the HBICA was terminated.

Rossman, however, continued to receive breeder queens of the most productive and desirable stocks from Madison in exchange for solicited evaluation of the stock from his Island Hybrid customers. After the closing of the Pelee Island facility in August 1965, Rossman continued to produce Island Hybrids from stock maintained at Madison. This outlet continued until 1970 when the USDA established the Honey Bee Stock Center at Baton Rouge, La.

Testing and Evaluating Hybrids and Inbreds

A vital part of any breeding and selection program is the continued testing of selections made before further effective breeding can continue.

Honey production

At Madison, 200 test colonies in four outyard locations were evaluated for honey production of the

stocks. All test colonies were requeened in late summer (August-September) with the queens to be tested. All tests ran 12 months to enable the queens to head full-strength colonies that were overwintered and managed intensively for honey production (*4*).

Sealed broods were measured for queen productivity and supporting populations. Package colonies, the most uniform in their initial populations, gave the best comparison between lines based on brood counts.

All test colonies were weighed in October and April of each year to make weight adjustments and to estimate weight gains and losses. In addition, all supers added or removed from the colonies were weighed and credited to the colonies. All pollen supplement or sirup fed was also recorded to give a complete weight record for each test colony through the year.

Each year the yield data on colonies tested were summarized, based on overall weight gain or loss October to October, minus 60 pounds required for winter stores. Because drifting of bees was early recognized as a factor in production studies, the test colonies were individually isolated to minimize drift.

In 1953, for ease of colony handling and to accommodate studies on mechanized hive handling, the four test yards were rearranged so that four isolated groups of colonies in each yard were arranged in straight rows. Within the rows drifting of bees was noted, though efforts were made to control drifting by hive markers, panels, and colors. For these reasons, test stock was arranged so that particular groups of hybrids appeared in each isolated straight line, with 10 to 13 colonies in a group. More credence was given to average yields within each stock group than to individual colony yields because of drifting of bees.

The four-block-per-yard arrangement allowed repetition of stocks in each of the four yards, so that four stocks replicated four items (in each of the yards) could be tested each season. When one superior hybrid was found (for example, the (ZX) (ME) in 1955), it could be repeated as the best stock for comparison in succeeding years. This best stock then occupied one block of colonies in each yard; three other prospective stocks were tested against it with the intent to use the best performer for a standard in succeeding years.

Evaluation of the contribution of inbred lines to honey productivity in hybrid combination was possible by preparing frequency distribution tables based on the relative production of the colonies in percentage of the maximum. In this, the more productive and least productive stocks were grouped so that the frequency of appearance of particular inbreds could be easily seen in high- or low-ranking hybrid combinations.

American foulbrood resistance

The main thrust of the USDA stock selection and breeding program was for high honey production. A good deal of attention was given to resistance to American foulbrood (AFB), especially in the early years of the breeding program. The Madison laboratory studied honey production and contributing traits while the Laramie, Wyo., laboratory tested each of the hybrids for AFB resistance.

The comb insert method was used at Laramie. A diseased section of comb was placed in the center of the brood nest of a test colony to study its effect on AFB. Some hybrids were found resistant and some were susceptible to AFB. Unfortunately, the resistant lines carried a good deal of temper and were discontinued.

Other characteristics

Records were made of temperament, behavior of bees other than temper (runniness, nervousness, flightiness), fall weight of brood nests, swarming tendency (indicated by swarm cell construction), disease susceptibility (both adult and brood diseases), propolization, bur and brace comb building, brood quality, conformation of queens, appearance of honey cappings, and body color plus any unusual characteristics, unique to a particular stock that were noted during the year.

Dr. Roberts made an extensive study of morphological characteristics of inbred and hybrid bees in 1953 and 1954 (*16*). Workers were examined morphologically for length of left tibia of the third leg, length of the second cubital cell of both right and left anterior wings, and number of wing hooks on each hindwing. Length of time for development was also studied.

Differences were found between the inbreds and hybrids in length of development, length of tibia, number of wing hooks, and number of ovarian tubules, but these were not significantly demonstrated. There was an indication that the inbred line X had a shorter second cubital cell than the other lines tested. Differences also appeared between inbred lines in mating and start of egg

laying, but again, the data were not conclusive. The gross weight of queens was apparently correlated with weight of ovaries. The weight of head and thorax was probably the least variable characteristic in the hybrids tested.

Comparison of the reciprocal hybrids Y × Z and Z × Y showed a slight advantage for the latter, but again, the data were not conclusive. Because egg nutrients from one inbred line may differ from that of another line, reciprocal hybrid queens may differ in their development. If such a maternal effect were true, better hybrid queens may be produced by a three-way cross or specific two-way crosses. The trend, however, has been to produce five- and six-way hybrids as it is more economical.

Data on production and general characteristics

Rather than summarizing 20 years of stock testing, data on honey production and general characteristics accumulated on innumerable hybrid and

TABLE 1.—*Net yields of colonies with original queens, 1953 (Stocks ranked on production by yards)*

Stock	Average production		Max.	Min.	Average fall weight brood nest	Max.	Min.
	Pounds[1]	*Percent*			*Pounds*[1]		
Slotten yard:							
(Z×X) × (E×P)	149 (11)	100	200	98	165 (11)	186	150
(X×M) × OAC	144 (2)	97	145	144	145 (2)	150	141
(X×M) × (E×P)	132 (4)	89	158	84	149 (4)	173	130
(W42×S) × (E×P)	118 (5)	79	156	65	141 (2)	150	132
(X×Y) × (E×P)	95 (6)	64	161	9	158 (3)	173	134
(T×N) × (E×P)	81 (4)	54	106	49	149 (2)	153	144
(X×S) × (M×E)[2]	61 (1)	41	—	—	153 (1)	—	—
Miscellaneous	59 (4)	40	104	30	146 (4)	153	123
Average	115 (37)				155 (29)		
Primrose yard:							
(Z×X) × (E×P)	226 (8)	100	264	157	161 (6)	168	144
(X×S) × (E×P)	180 (8)	80	230	151	169 (7)	189	142
(T×Y) × (E×P)	177 (6)	78	209	159	154 (6)	183	140
(T×N) × (E×P)	175 (7)	77	195	152	146 (6)	157	133
(Y×S) × (E×P)	171 (10)	76	202	143	153 (8)	166	143
(X×S) × (M×E)[2]	115 (4)	51	172	88	174 (4)	190	157
Miscellaneous	93 (3)	41	110	79	147 (2)	169	125
Average	174 (46)				158 (39)		
Zweifel yard:							
(Y×S) × (E×P)	193 (8)	100	232	143	118 (7)	141	103
(Z×S) × (E×P)	149 (8)	75	172	65	120 (6)	140	90
(W42×S) × (E×P)	147 (9)	74	193	87	116 (6)	138	95
(X×Y) × (E×P)	132 (7)	66	209	71	143 (7)	169	124
(N×Y) × (E×P)	105 (10)	53	137	79	99 (10)	120	89
Miscellaneous	90 (4)	45	156	—30	130 (3)	145	115
Average	140 (46)				119 (39)		
Zurbuchen yard:							
(X×M) × (E×P)	195 (1)	100	—	—	201 (1)	—	—
(X×M) × OAC	148 (4)	76	178	97	191 (2)	200	142
(Z×S) × (E×P)	147 (7)	75	224	104	158 (3)	163	150
(X×S) × (E×P)	133 (7)	68	172	93	196 (6)	233	172
(S×X) × (M×E)[2]	127 (7)	65	168	78	192 (2)	204	180
(N×Y) × (E×P)	123 (10)	63	237	43	169 (9)	215	132
(T×Y) × (E×P)	122 (4)	63	151	47	178 (2)	187	169
Miscellaneous	80 (4)	41	108	23	151 (1)	—	—
Average	129 (44)				179 (26)		

[1]Number of colonies comprising averages indicated by parentheses.
[2]Commercial breeder.

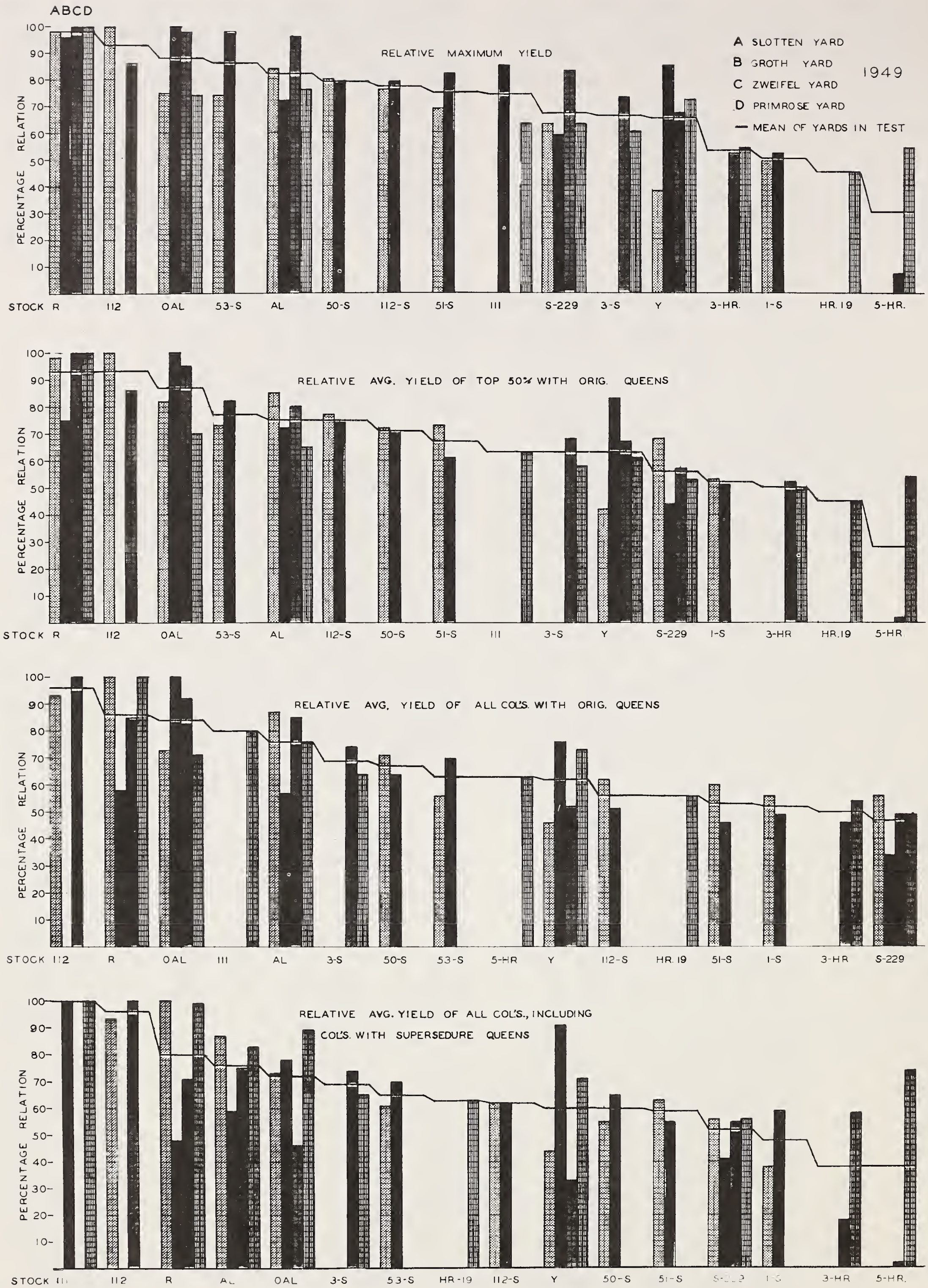

FIGURE 1.—Stock testing production data for 1949.

inbred combinations are presented in tables 1 to 6.

Figure 1 summarizes production data for 1949, when stock R was the best performing hybrid—top yielding colony in one yard exceeded 200 pounds. Stocks tested that year are shown ranked on average yields converted to a percentage of the maximum (shown as 100). This ranking was done each year for all stocks tested until 1953, when stocks were rearranged into straight-row test blocks.

Table 1 summarizes production data for 1953 and gives the raw data from which relative yield comparisons, such as those shown in figure 1, can be calculated. The average fall weights of brood nests for which good overwintering colonies must be selected are also included in table 1. Colonies to overwinter properly in northern areas must be selected for compact brood nests well provisioned with honey stores in late fall.

A high degree of correlation exists between the fall weight of brood nests and honey production in the subsequent season. Correlation, however, is less striking when the main honey flows come late in the summer. Table 2 illustrates this correlation for 2 years—by stocks in 1951 and by yards over all stocks in 1954.

Table 3 summarizes production data for 1959 when (ZX) (ME) stock was the best performing hybrid, the average yield for 2 yards exceeding 175 pounds.

Table 4 illustrates how general behavior characteristics, including relative production rank, were used to further evaluate various stocks. These stocks were all closely related. The X_z line is a reconstituted X line containing 15.6 percent Z. The ZX or reciprocal XZ hybrids all tend to have heavy brood nests at the end of the season and have minimum swarming tendencies.

An index of the inbred stocks contributing most to high production in hybrid combination was made possible by calculating a frequency distribution when all stocks were mated to the same drone type as occurred in the queens produced on Pelee Island (table 5). Inbreds appearing most frequently in the more productive stocks were D, G, H, N, and Z. Lines S and X appeared frequently throughout the range, depending on the particular combinations

TABLE 2.—*Correlation between fall weights of brood nests and production of honey during the subsequent season*

Stock and yard		Average gross weight of brood nest	Average net yield of honey	Correlation r
	Number	*Pounds*	*Pounds*	
Stock, 1951:				
A	7	91	63	[1]0.8844
B	8	117	158	.6129
C	6	117	202	[1].9840
D	6	102	171	[1].9195
E	4	125	207	[1].9789
F	4	120	186	.8710
All	35	111	159	[1].8192
Yard, 1954:				
1	34	153	110	[1]0.5459
2	36	155	176	[2].4013
3	36	118	141	.2787
4	25	178	135	[1].7198

[1]Significant at the 0.05 percent level of probability.
[2]Significant at the 0.01 percent level of probability.

TABLE 3.—*Surplus honey, 1959 stock testing*

Stocks[1]	Yield[2] Maximum	Minimum	Average		Rating
	Pounds	*Pounds*	*Pounds*[3]		*Percent*
Slotten yard:					
(ZX)(ME)	262	100	182	(7)	100
(X_zZ)(ME)	211	86	166	(8)	91
(ZS)(ME)	109	64	92	(6)	51
(Z2)(ME)	97	33	65	(2)	36
All stocks	262	33	142	(23)	
Primrose yard:					
(ZX)(ME)	214	148	177	(4)	100
(Z2)(ME)	173	39	89	(3)	50
(ZS)(ME)	131	17	82	(5)	46
(X_DZ)(ME)	67	64	66	(2)	37
All stocks	214	17	108	(14)	
Zweifel yard:					
(ZX)(ME)	181	113	146	(6)	100
Package bees source 1[4]	138	30	96	(8)	66
Package bees source 2[4]	116	4	51	(10)	35
(ZS)(ME)	98	−32	41	(7)	28
All stocks	181	−32	79	(31)	
Groth yard (package yard):					
Source 1 (treated)[4]	125	9	75	(11)	100
Source 2 (treated)[4]	94	−3	51	(9)	68
Source 1 (untreated)[5]	69	21	46	(10)	61
Source 2 (untreated)[5]	49	−21	21	(8)	28
All stocks	125	−21	50	(38)	
All yards	262	−32	86	(106)	

[1]Stock lines ranked on basis of relative production in each yard (all colonies with original queens).
[2]Package yields—April to October gain less 60 pounds.
[3]Number of colonies are in parentheses.
[4]Treated packages received 1½ gallons Fumidil B sirup (150 mg fumagillin) during the first 3 weeks after installation.
[5]Untreated packages received 1½ gallons unmedicated sugar sirup.

Table 4.—*Summary of stock characteristics, 1959*

Stock	Relative production	Temper	Weight brood nest	Handling qualities	Swarming tendency	Bur comb	Original queen survival
		Behavior characteristics[1]					
	Pounds[2]						*Percent*
(X$_z$Z)(ME)	100 (10)	10	10	9	8	9	70
(ZX)(ME)	99 (26)	10	10	9	8	8	70
(X$_z$Z)(M1)	61 (32)	10	10	9	9	9	90
(Z2)(ME)	52 (18)	10	9	8	3	8	30
(ZS)(ME)	41 (20)	10	6	8	5	6	90
Commercial	38 (32)	8	8	6	9	3	90
(X$_D$Z)(ME)	37 (8)	10	10	9	8	9	30

[1]Behavior characteristics are given as abstract values rated from 10 to 1 in descending order of merit.

[2]Number of colonies represented, including original and supersedure queens, are in parentheses.

represented. Lines P, W, and Y appeared most frequently in the less productive hybrids.

The performance of Island Hybrid queens sold to commercial beekeepers was followed annually by solicited information obtained from the beekeepers

Table 5.—*Relative production of stocks grouped by yards*[1] *and frequency of appearance of inbred lines; queens all mated to M×E drones, 1954*

	Average	Relative production (percent of yard maximum[2]) 50-70	71-80	81-90	91-100
Yard:					
Primrose	202	Y×S	G×S	G×X	
				S×X	
Zurbuchen	178	P×X	D×X	Z×X	
		Y×X			
Slotten	167	W×S		Z×S	X×S
		W×X			N×X
					X×Z
Zweifel	157	Z×S		H×S	D×S
		Frequency of appearance			
Inbred line:					
D			1		1
G			1	1	
H				1	
N					1
P		1			
S		3	1	3	2
W		2			
X		3	1	3	3
Y		2			
Z		1		2	1
Total		12	4	10	8

[1]Based on average production of colonies with original queens in early July.

[2]Yard maximum refers to the top yielding colony in the yard.

Table 6.—*Evaluation of commercial hybrids (ZX) (M1) and (6ZX)(M1) by 42 commercial beekeepers compared with other stocks used in 1962*

(Percentage of 2,489 queens based on beekeepers reporting on each category)

Characteristic	General ratings, 1962: Inferior	Average	Above average	Superior	General rating above average or superior: 1962	1961
Production	4	20	50	26	76	94
Brood rearing	9	18	51	22	73	95
Nonswarming	0	31	54	15	69	78
Temperament	5	43	24	29	52	69
Weight of winter brood nest	5	37	39	18	58	80
Less bur comb	10	63	20	7	27	30
Less propolis	19	46	24	11	35	39
Supersedure	11	32	38	19	57	27

through standardized forms. Table 6 summarizes the reports of 42 beekeepers of 85 who purchased the hybrid stock (ZX)(MI) in 1961 and 1962. This summary represents 2,489 queens. Such information in general agreed with test observations made at the lab. Six others who purchased 273 queens made special favorable comments without attempting to rate the stock specifically. Several beekeepers wrote special letters of commendation.

Selected examples of stock variability

Throughout the 20 years of stock testing at Madison, researchers noted a great variety of expressions of stock variability in honey bees.

Temperament is apparently governed by a multiple-gene effect. It varies greatly, as most experienced beekeepers know well. Some strains of bees are mean only under certain local weather or climatic conditions, and are sensitive to temperature change, light change, or nectar flows. Degrees of temper vary from slight, to mean, to vicious.

Some stocks selected for American foulbrood (AFB) resistance, notably (A18 x Bur) carried excessive viciousness. Apparently some genes responsible for AFB resistance must be close to the genes responsible for temper. In 1951, when this drone source was used on Kelleys Island, all the resultant test queens produced vicious offspring. Colony manipulations that year had to be modified. Bee gloves and coveralls, frowned on by progressive beekeepers as cumbersome and unnecessary, had to be used even during the height of the honey flow to avoid unbearable punishment. Farmers in

fields up to one-fourth mile away were frequently stung. On another occasion during 1948, one colony of highly productive stock was so gentle that one could get no deliberate stings, regardless of how rough the bees were treated. Experience has shown that there is no correlation whatever between honey productivity and colony temper.

General bee behavior aside from temper was noted. "Runniness" or general nervousness is distinct from temper. Likewise, bees can be nervous and jittery on the combs without being runny or temperamental. Conversely, some strains may be quiet and almost motionless even as the combs are removed and inspected. Strains were observed that were flighty. Such bees would take to the air in great numbers as the colony was being manipulated. This behavior, combined with the jittery tendency and adverse temper, can produce vicious bees. Some strains were observed that simply ran from the combs so severely that when the hive cover was removed, combs could be taken out with scarcely a bee on them. A little smoke would send virtually all the bees out of the hive and onto the ground.

One strain of bees was seen that simply refused to draw or build comb on wax foundation given to them. They would chew the foundation out and build comb of their own without the foundation as a guide.

Another hybrid refused to cap honey in the comb even after it was fully ripened, except after crowding of storage space.

Recently we had a hybrid that produced excessive wax. Gobs of wax were plastered over all the inner surfaces of the hive, and much bur and brace comb were constructed everywhere.

The appearance of capped honey also varied greatly. The cappings produced by some strains were white because of an air space under the cappings. In others the honey was in contact with the cappings, which gave a wet appearance to the finished comb. The smoothness or finish of cappings themselves varied greatly.

In 1950, the A18 Gaf × S10 W 39 hybrid showed susceptibility to paralysis disease. In one test yard, when paralysis appeared in these colonies, excellent populations were virtually decimated as if by pesticide poisoning within 10 days to 2 weeks during May. Pounds of dead and dying bees piled up at the hive entrances. Other colonies of different hybrids in the same yard were not susceptible and showed no symptoms.

Selection of the best stocks

Over the years hybrids were constructed that were superior to the best we have today. These were lost mainly because of the loss of their constituent inbreds. The R stock of 1949, one of these hybrids, was probably superior to the popular (BZX)(MD). Preservation of inbreds has been the weak point in the system of inbreeding and hybridization.

Our best hybrid, (BZX)(DM), is a selection made from present stocks. The (ZX)(ME) stock, selected in 1955, was gradually changed because of the loss at intervals of the constituent inbreds that each time were "reconstituted" by using naturally mated sister inbreds to provide drones for repeated backcrosses of daughter queens. This process started as a brother-sister mating from the open-mated inbred to produce a daughter to be again mated back to "uncle" drones of the original open-mated inbred to produce a third and fourth generation, each time mating back to the original queen's drones. After about four generations of such uncle-niece matings, we arrive at a reconstituted inbred.

The original (ZX)(ME) was highly productive; produced no bur comb; and had a moderate degree of temper, heavy fall brood nests, minimum swarming tendency, minimum propolization, good overwintering, and good white cappings.

Like the original (ZX)(ME) hybrid, (BZX)(DM) is also highly productive, has heavy fall brood nests, minimum swarming, minimum propolization, good overwintering, white cappings, gentle bees, but has moderate bur combing tendency. Queens are black, Caucasion-type; and after crossing to the Italian-type DM drones, offspring are dark banded.

The Z is a black Caucasian-type stock, highly inbred, from a line begun in 1952 from stock obtained from N. R. Chamberlin, Poplar, Wis. In 1958, some crossing with the X line resulted in about 10 percent X being incorporated into Z.

The X line is a black Caucasian line, highly inbred, and carries some temper and susceptibility to sacbrood. It began in 1952 from stock from Baton Rouge that came from C. G. Butler in England. The line has about 15 percent Z.

The B line, a dark-black Caucasion, will uncap AFB brood. This line was started in 1955 from Caucasian stock obtained from the Bolling Bee Company in Alabama.

The M line is a light cordovan inbred begun in 1946 from a light selection from a Caucasian-Moore naturally mated queen and will also uncap AFB brood. The D line is a yellow selection started in 1957 from stock obtained from Dyment Brothers in Smithville, Ontario, Canada.

Honey bee stocks cannot be shipped from one country to another in the adult form without the danger of transmitting adult bee diseases, mainly *Acarapis woodi,* which causes acarine disease. Dr. M. V. Smith of Guelph, Ontario, Canada, perfected an incubator that kept honey bee eggs, larvae, and pupae alive and in good condition during a journey of 20 hours (*19, 20*). He was thus able to legally import these immature forms from England without accompanying adults.

Commercial hybrid breeding

Following the success in breeding bees by the USDA and Canada, the firm of Dadant and Sons, Inc., entered the field and is highly successful in the commercial production of hybrid queens. Their program was headed by Dr. G. H. Cale, Jr., (*1, 2*) who took his training in artificial insemination under Dr. W. C. Roberts at Madison.

Problems encountered and loss of past hybrids

Selection, inbreeding, and crossing have been the basic tools in hybrid breeding of honey bees. Success has been outstanding, but a basic problem concerns maintaining the inbred lines. To retain superior hybrids, the component inbreds must be maintained and crossed each time the superior hybrid is to be reproduced. Unfortunately, the inbreds are usually inferior as queens to head up colonies. All too often they tend to be superseded unless a great deal of attention is given to removing queen cells periodically. Inbred populations are usually inferior, and as a result such units are difficult to overwinter unless supported by the addition of more bees from hybrid colonies. Winter loss is frequently high. To circumvent overwintering problems, inbred queens can be held in small units above full-strength hybrid colonies to benefit from the heat of the colony below, as well as receive upward drift of bees.

Research is continuing at Madison to perfect a method of banking or overwintering large numbers of queens with a minimum of loss. Some success has been achieved (*5*), but generally such methods are not yet sufficiently dependable to preserve valuable inbred stocks.

A problem encountered in hybrid queen production is the insured availability of sufficient grafting material for commercial production. This supply has been maintained to a large extent by the use of a three-way hybrid queen mother giving a five-way hybrid as the final product. Such queens assure better and more vigorous colonies for grafting material.

A Look to the Future

Bee breeding is an effective method of producing productive hybrids and gentle stocks. We have only begun in the area of genetic improvement and selection. A line of alfalfa pollen-collecting bees has been developed (*2, 13*). Unpublished work in Wisconsin has shown that bees can also be selected for cranberry pollen-collecting tendencies which is a heritable trait. With techniques available selecting and breeding for virtually any trait desired in honey bees is now probable. Thus, we could have tailor-made bees for any purpose.

The predicted appearance of the Africanized honey bees of Brazil in this country would make the hybridization with and preservation of gentle honey bee strains highly important. As new methods of queen and drone production are developed and advances are made in artificial mating and handling of queens to insure safe introduction into colonies, natural matings may soon be entirely replaced by controlled bee breeding and hybridization. The need to control Africanized bees may hasten this development.

Much effort in research is directed toward methods of preserving breeding material. Research is progressing on queen and colony storage, and study is in progress on methods of storing sperm.

Sperm storage is an area of research that must be exploited. If sperm storage were possible, inbred line maintenance could be greatly facilitated.

In 1959, 10 queens were inseminated at Madison with semen that had been collected by S. Taber, III, of the Baton Rouge Laboratory and sent to Madison by mail (*22, 23*). The semen was sealed in four glass tubes. About 3 mm^3 was given each queen; all queens except one that died started laying promptly. One produced a mixture of worker and drone brood. All others produced 100 percent

worker brood. Little further research on sperm storage or shipment has been done since that time except by Poole and Taber (*14*).

The method of producing hybrid bees has been inbreeding, selection, and hybridization. Other improved breeding patterns should be considered to avoid reaching high levels of inbreeding in the base stocks. One such plan is a circular breeding system suggested in 1974 by Dr. W. C. Roberts (*18*). This would utilize as many as 10 or 12 families of unrelated strains of bees that would be crossed with each other over several years.

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